Hemoglobin Synthesis in a Cell-free System*

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ABSTRACT: Hemoglobin synthesis was studied in the rabbit reticulocyte cell-free system of R. Schweet, H. Lamfrom, and E. Allen (1958, *Proc. Natl. Acad. Sci. U.S. 44*, 2163). In this system, amino acid incorporation proceeds at about 1% the whole cell rate of 100 amino acids per minute per ribosome. The amino acids are added stepwise to incomplete polypeptide precursors found in the ribosomes; chain growth proceeds from the amino to the carboxyl terminus. Finished chains are released from the ribosomes without any appreciable delay. However, the initiation of new chains is essentially absent. One complete hemoglobin chain is released per three to four ribosomes in the most active preparations. Thus, about half of the ribosomes which were participating in protein synthesis in the whole cell can complete and release their polypeptide chains during the cell-free incubation. On the average, 50% of each chain released is composed of amino acids added during the cell-free incubation. Hence, the chains which are completed are selected, without regard to their initial length, from the total polypeptide chain population present on ribosomes prepared from the whole cell.

Studying the *in vitro* synthesis in cellular systems of proteins of known amino acid sequence has provided the best evidence for the mechanism of polypeptide chain formation (Dintzis, 1961; Naughton and Dintzis, 1962; Canfield and Anfinsen, 1963; Luck and Barry, 1964). After short incubation times with labeled amino acids it has been shown that the radioactivity is distributed nonuniformly along the polypeptide chains, indicating that the chains are assembled by the sequential addition of amino acids, starting at the NH_2-terminus. The enzymatic steps of peptide bond formation are now being studied in detail (Arlinghaus *et al.*, 1964; Traut and Munro, 1964).

The distribution of radioactivity among the tryptic peptide fragments of hemoglobin isolated from ribosomal particles after pulse-labeling of rabbit reticulocytes has also been studied (Dintzis, 1961). The results indicated that the hemoglobin chains are formed on the ribosomes. It has since been shown that the ribosomes active in protein synthesis are present in the polyribosome structures of the cells (Marks *et al.*, 1962; Warner *et al.*, 1963; Gierer, 1963). The incomplete polypeptide precursors are found linked to transfer RNA molecules at their growing points (Takanami, 1962; Gilbert, 1963; Bretscher, 1963; Arlinghaus *et al.*, 1964).

We have investigated the synthesis of hemoglobin chains in the rabbit reticulocyte cell-free system (Schweet *et al.*, 1958) in order to evaluate the intactness of the protein synthetic machinery after fractionation of the whole cells. The process of amino acid assembly was studied in detail and a comparison with the whole cells was made with respect to the rate of chain growth, initiation of new chains, release of completed chains, and the number of ribosomes participating in the process.

Materials

The sodium salts of adenosine triphosphate, guanosine triphosphate, and phosphoenolpyruvate were obtained from the California Corp. for Biochemical Research. Pyruvate kinase was purchased from C. F. Boehringer and Son, Mannheim, Germany. DL-[4,5-^{3}H]Leucine was a product of the New England Nuclear Corp. and had a specific activity of 3.6 mc/μmole. Uniformly labeled [^{14}C]leucine (6–8 mc/mmole) was obtained from Nuclear Chicago Corp. Millipore filters were obtained from the Millipore Filter Corp. and had a diameter of 25 mm and a 0.35-μ pore size. CM-cellulose was obtained from the Brown Co., Berlin, N.H. (capacity 0.47 meq/g).

Methods

The cell fractions were prepared from rabbit reticulocytes according to the procedures described by Schweet *et al.* (1958). The reaction mixture contained[1] (in 1 ml): $MgCl_2$ (4 μmoles), ATP (1 μmole), GTP (0.25 μmole), PEP (10 μmoles), pyruvate kinase (50 μg), an amino acid mixture minus leucine (Borsook *et al.*, 1952), radioactive

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[1] Abbreviations used in this work: ATP, adenosine triphosphate; GTP, guanosine triphosphate; PEP, phosphoenolpyruvate.

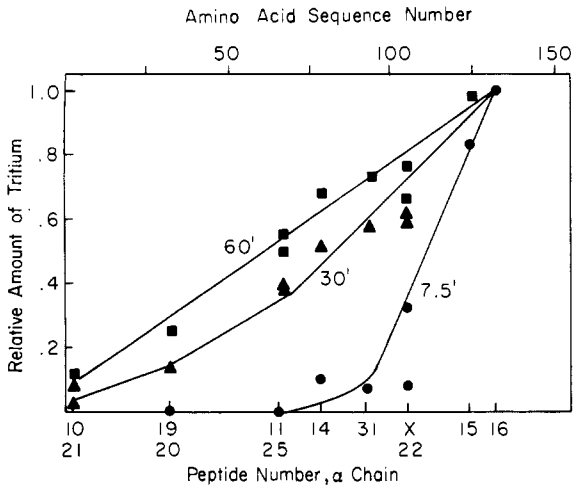

FIGURE 1: Distribution of [^{3}H]leucine among the tryptic peptides of the soluble hemoglobin (α-chain) after various incubation times in the cell-free system at 37°. A description of the analysis procedure is given under Methods. The peptides have been placed according to the leucine positions of the corresponding peptides in human hemoglobin. There are 141 and 146 amino acid residues in the α- and β-chains, respectively; amino acid 1 corresponds to the NH_2-terminus.

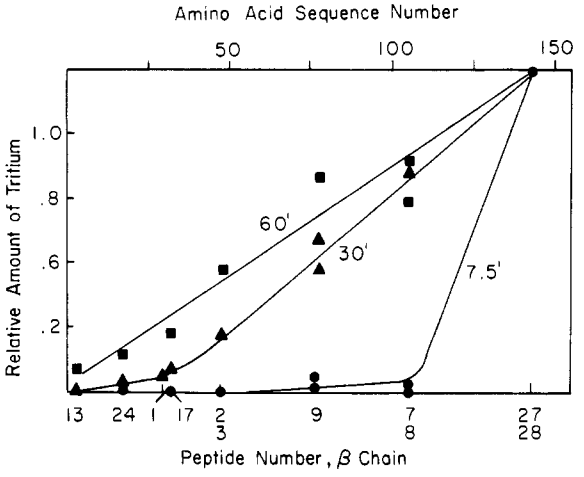

FIGURE 2: Distribution of [^{3}H]leucine among the tryptic peptides of the soluble hemoglobin (β-chain) after various incubation times in the cell-free system at 37°. See legend to Figure 1 for details.

leucine (0.1 μmole), Super I[2] (0.1 ml), *p*H 5 fraction (5–6 mg protein), and ribosomes (3–6 mg). The *p*H 5 fraction was dissolved in 0.1 M Tris-HCl buffer, *p*H 7.5, and the ribosomes were in medium A (Keller and Zamecnik, 1956). The ribosome concentration was determined by optical absorbance measurements at 260 mμ (T'so and Vinograd, 1961).

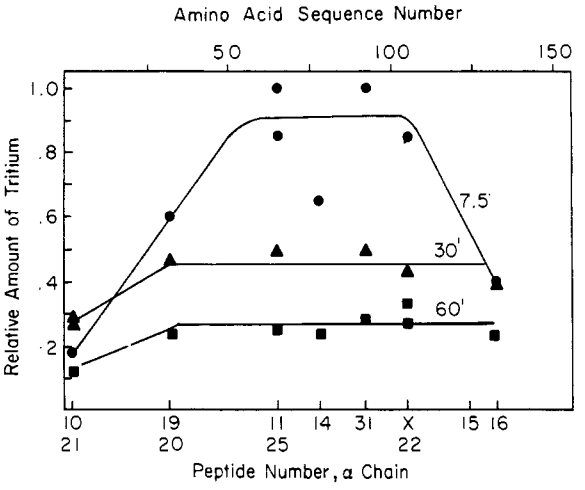

FIGURE 3: Distribution of [^{3}H]leucine among the tryptic peptides of the ribosomes (α-chain) after various incubation times in the cell-free system at 37°. See legend to Figure 1 for details.

[^{14}C]Leucine was the label used to study the general properties of the cell-free system. After incubation at 37°, samples from the reaction mixtures were precipitated with trichloroacetic acid at a final concentration of 3.5% (w/v) and treated by the method of Siekevitz (1952). The precipitates were collected on preweighed Millipore filters and all samples were air-dried, weighed, and then counted, by means of a Nuclear Chicago end-window gas-flow counter. The results were corrected for self-absorption to zero thickness and expressed in terms of cpm/mg ribosomes. The statistical uncertainty of the method described is ±15%.

The kinetics of incorporation into the ribosomal and supernatant proteins were studied by taking samples (0.25 ml) at specified times, adding them to 8 ml of a 5 mM $MgCl_2$ solution at 0°, and then centrifuging down the ribosomes after all the samples had been collected. The supernatant fractions were prepared for counting as described above. The centrifuge tubes containing the ribosome pellets were quickly rinsed twice with cold medium A and the pellets were resuspended in water. Super I was added as a carrier and the samples were prepared for counting.

The process of polypeptide chain formation was studied by using [^{3}H]leucine as the label in a 7-ml incubation mixture. After incubation the ribosomes were removed and washed three times by centrifugation, each time in 30 ml of medium A at 100,000 × *g* for 1 hour. Prior to the final high-speed centrifugation, the ribosome samples were centrifuged at 15,000 × *g* for 15 minutes to remove the insoluble material. The distribution of [^{3}H]leucine among the tryptic peptides of the soluble hemoglobin and ribosomes was determined by methods previously described (Dintzis,

[2] The supernatant from the 100,000 × *g*, 1-hour centrifugation of the cell lysate (Schweet *et al.*, 1958).

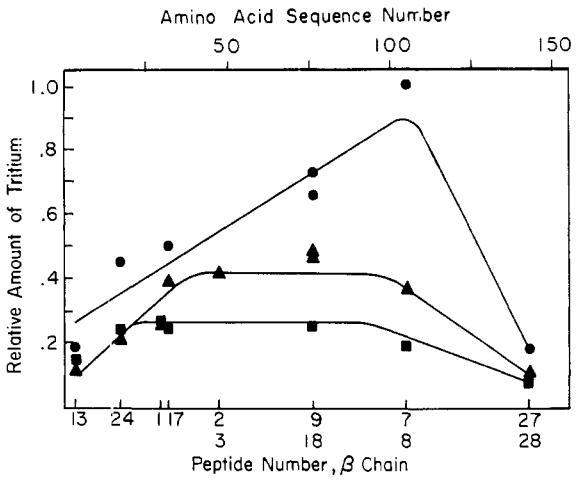

FIGURE 4: Distribution of [^{3}H]leucine among the tryptic peptides of the ribosomes (β-chain) after various incubation times in the cell-free system at 37°. See legend to Figure 1 for details.

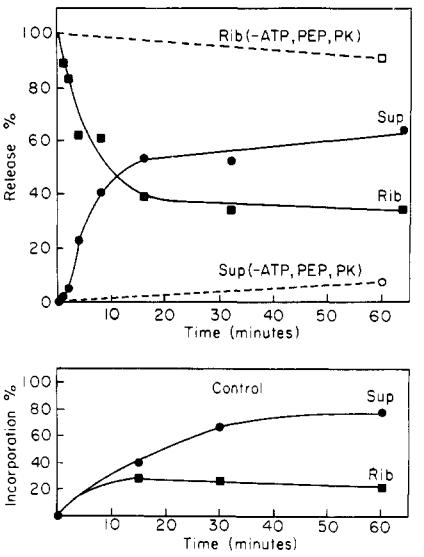

FIGURE 5: Kinetics of the cell-free system. (a) Kinetics of release of radioactivity in the cell-free system from ribosomes prelabeled by incubation of intact reticulocytes with [^{14}C]leucine. The complete system was incubated at 37° and samples were prepared for analysis as described under Methods. The results are expressed as per cent of total radioactivity in the ribosomes at 0 minutes (100% = 0.3 mμmole leucine/mg ribosomes). PK represents pyruvate kinase. (b) Kinetics of incorporation of [^{14}C]leucine into the ribosomal and supernatant fractions of the cell-free system. The ribosomes were prepared from intact reticulocytes which had been incubated with unlabeled leucine; otherwise all components were the same as used for the release experiment described in (a). The results are expressed as per cent of total radioactivity incorporated at 60 minutes (100% = 0.6 mμmole leucine/mg ribosomes).

1961). The tritium-labeled samples were mixed with hemoglobin uniformly labeled with [^{14}C]leucine and digested with trypsin. For the analysis of the soluble hemoglobin fraction, the α and β polypeptide chains were separated by column chromatography prior to digestion (Dintzis, 1961). The tryptic digests were fractionated by performing high voltage electrophoresis and chromatography at right angles on paper ("fingerprinting"), using the high-loading technique described by Naughton and Dintzis (1962). The tryptic peptides were eluted from the papers after ninhydrin staining and the radioactivity was measured with a Packard Tri-Carb liquid scintillation counter. For the analysis of ribosome digests, a third dimension of purification of the peptides in the fingerprint was performed by means of an additional electrophoretic separation (Naughton and Dintzis, 1962). This procedure partly overcame the variability found previously in ribosome analyses (Dintzis, 1961).

The release of polypeptide chains during the cell-free incubation from ribosomes labeled in the whole cell was studied (and referred to as the "reverse-labeling" experiments in the text). Ribosomes were prelabeled by incubation of intact reticulocytes with radioactive leucine. Washed cells were suspended in a medium containing glucose, ferrous ammonium sulfate, sodium bicarbonate, and an amino acid mixture minus leucine at the concentrations described by Borsook and coworkers (1952). The mixture was incubated at 37° for 10 minutes, radioactive leucine was then added, and the incubation was continued for 10 minutes. It was stopped by adding an ice-cold saline solution (0.13 M NaCl, 4.5 mM KCl, 7.5 mM $MgCl_2$) and the cells were washed twice by centrifugation. The cells were lysed and the ribosomes isolated as before. The *p*H 5 fraction and Super I were prepared from unlabeled cells. The other components were added to the cell-free incubation mixture in the concentrations previously described except that nonradioactive leucine was used. The kinetics of release of radioactivity from the ribosomes in the cell-free system were studied by the same methods as were used for the incorporation kinetics.

Results

General Properties of the Cell-free System. The requirements for incorporation of [^{14}C]leucine into the hot trichloroacetic acid-insoluble fraction were similar to those observed by other investigators (Keller and Zamecnik, 1956; Schweet *et al.*, 1958). The total incorporation of [^{14}C]leucine in a 60-minute incubation varied in different preparations, from about 860 to 3800 cpm/mg ribosomes, and corresponded to 0.5–3 leucine residues/ribosome.

Between 50 and 80% of the [^{14}C]leucine incorporated in a 60-minute incubation appeared in the soluble fraction, the remainder being found in the ribosomes. The [^{14}C]leucine was incorporated initially into the ribosome fraction with no time lag and was detectable in the

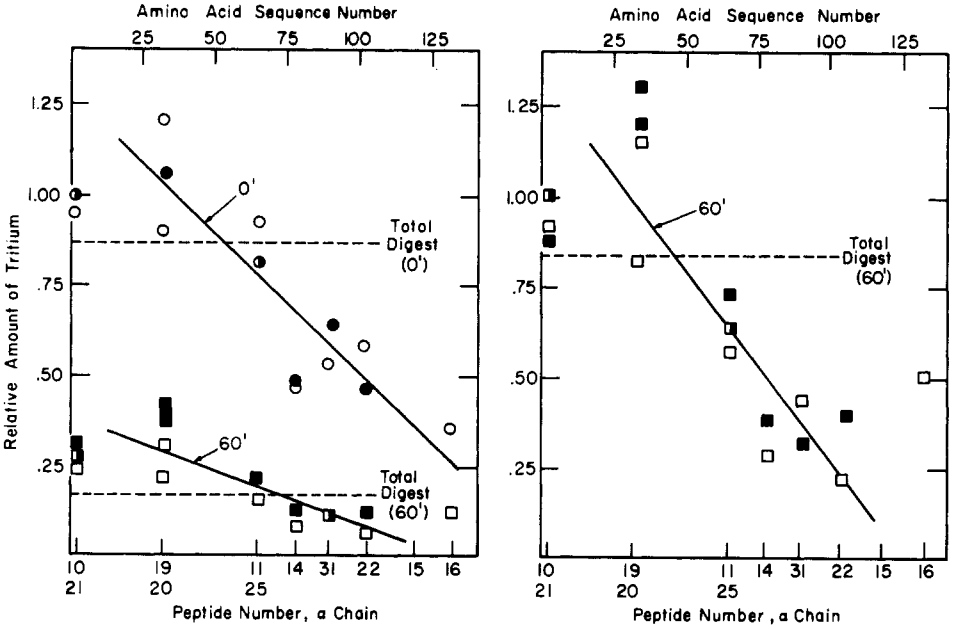

FIGURE 6: Distribution of [^{3}H]leucine among the tryptic peptides of the ribosomes (α-chain) at 0 and 60 minutes. Ribosomes prelabeled by incubation of intact reticulocytes were employed in the cell-free system at 37°. A description of the analysis procedures is given under Methods. The relative amounts of ^{3}H in the total digests are indicated on the graphs. Since hemoglobin uniformly labeled with [^{14}C]leucine was added in proportion to the mass of ribosomes used in each analysis, the 0- and 60-minute data are comparable on the same graph. The 60-minute data is also plotted on an expanded scale in the right-hand graph. Two separate analyses were performed for each sample and are indicated as the open and closed symbols.

supernatant fraction after 2–4 minutes of incubation. The maximum initial rate of total incorporation found was between 1.0 and 1.5 amino acids/ribosome per min—about 1% of the rate for the intact reticulocytes (Dintzis, *et al.*, 1958). We have assumed here that 10% of the amino acid residues in rabbit hemoglobin are leucine (see Discussion).

Polypeptide Chain Assembly. Cell-free incubations were performed with [^{3}H]leucine and samples were withdrawn at various times. The ribosomes were separated from the supernatant fraction by centrifugation. The samples were prepared for analysis after having been mixed with hemoglobin uniformly labeled with [^{14}C]leucine. Tryptic digests were "fingerprinted" and the radioactivity in the peptides determined (see Methods). The results of these experiments are given in Figures 1–4. The tryptic peptides of the rabbit α- and β-chains have been arranged into a sequence from amino to carboxyl terminus by Naughton and Dintzis (1962). The arrangement was based on the close homology in amino acid composition between the tryptic peptides of rabbit and human hemoglobin (where the amino acid sequence is known). The position of each peptide corresponds to the position of the leucyl residue in human hemoglobin. Peptides containing more than one leucine residue were placed at the numerical average of the leucine positions. Peptides 15, 16, 22, and X in the α-chain have been positioned near the carboxyl end by virtue of their relative ratios of ^{3}H to ^{14}C.

SOLUBLE HEMOGLOBIN. From the labeling pattern as a function of time it can be concluded that the leucines incorporated are distributed in a nonuniform manner throughout the polypeptide chains (Figures 1 and 2). The gradient is steepest at the shortest time, with a number of peptides containing no detectable [^{3}H]-leucine. By 60 minutes, when the system had ceased to incorporate radioactive amino acids, a gradient of labeling is still quite evident with very little tritiated leucine in the amino-terminal peptide of each chain (peptides 10 and 21 of the α-chain and 13 of the β-chain). The results are consistent with the interpretation that amino acids are added stepwise to incomplete polypeptide chains on the ribosomal templates, proceeding in the direction from the amino to the carboxyl end. With one important exception, this resembles hemoglobin synthesis in the whole cell. In the cellular system, at 15°, essentially uniform labeling was obtained after 60 minutes (Dintzis, 1961). Here, even at 37°, uniform labeling is not found. We interpret this as meaning that few, if any, new polypeptide chains are begun. This conclusion is in agreement with the data reported by Bishop *et al.* (1960), who found little or no [^{14}C]valine incorporated into the NH_2-terminal valine positions of the chains during cell-free incubations.

At 60 minutes, in each peptide chain, the labeling pattern can be approximated by a straight line extending from the COOH-terminal to the NH_2-terminal

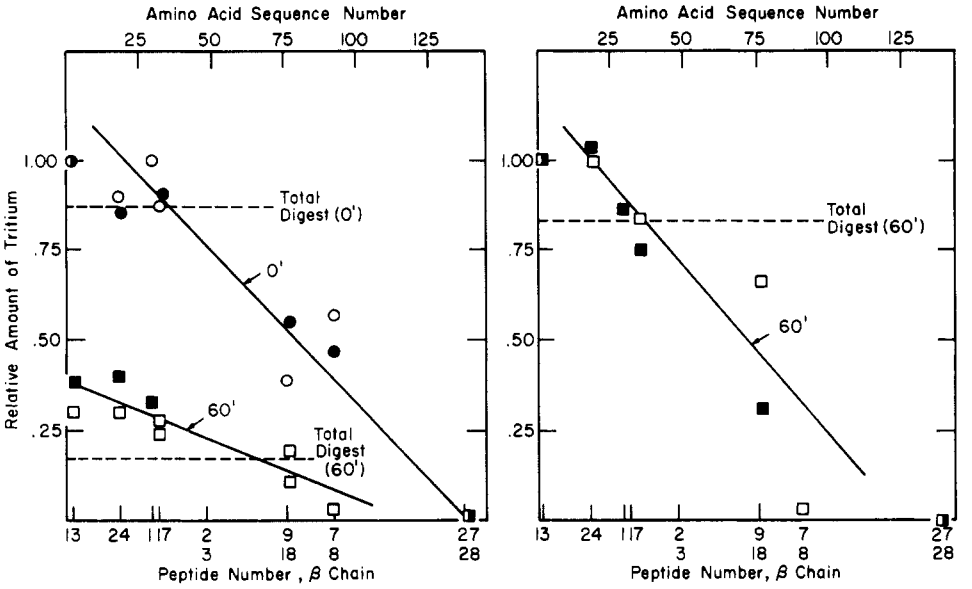

FIGURE 7: Distribution of [^{3}H]leucine among the tryptic peptides of the ribosomes (β-chain) at 0 and 60 minutes. See legend to Figure 6 for details.

(Figures 1 and 2). Hence it may be concluded that polypeptide chains of any initial length (at $t = 0$ min) have an equal probability of being completed and released from the ribosome during a 60-minute incubation.

At 7.5 minutes the labeling patterns observed indicate that some chains which have incorporated 40 amino acids (counting in from the carboxyl terminal) have appeared in the soluble fraction.

RIBOSOMES. The labeling patterns at short times (Figures 3 and 4) clearly show a gradient from the amino end into the interior of the peptide chains. A lack of initiation of new polypeptide chains, i.e., of growth starting with the NH_2-terminal amino acid, will produce such a result. In contrast, when whole cells are incubated, a uniform labeling pattern is observed at short times in the ribosomes (Dintzis, 1961). The falloff in the amount of ^{3}H at the carboxyl terminus (also observed by Dintzis) is due to the release of completed chains. It indicates very little delay between completion of the polypeptide chain and its release from the ribosome.

At 30 and 60 minutes, when incorporation is completed, the results show almost uniform labeling of the polypeptide chains remaining in the ribosomes. Two possible explanations for this observation are that (a) polypeptide chains which had a rather short initial length (approximately 25 amino acids or less) have been completed, or nearly so, but not released from the ribosomes, or (b) polypeptide chains of varying initial length have increased in size by a few amino acids during incubation and remained in the ribosome fraction.

The gradients from the amino terminus at 7.5 minutes result from the addition of some 30 to 40 amino acid residues per growing polypeptide chain. From the soluble hemoglobin analysis above, it was observed that up to 40 amino acids are added per chain by this time. Taking these observations collectively, one can conclude that an average of 35 to 40 residues are added per chain in 7.5 minutes, a rate of about 5 amino acid residues/polypeptide chain per min. This rate is roughly 3–4 times the initial rate of amino acid incorporation per ribosome (1–1.5 amino acid residues/ribosome per min). This discrepancy between the rate per chain and the rate per ribosome suggests that not all the ribosomes are equally active in protein synthesis.

Kinetics of Release. Ribosomes labeled in the whole cell for 10 minutes with [^{14}C]leucine were incubated in the cell-free system. Release of radioactivity from the ribosomes was measured and the results of such an experiment are shown in Figure 5a. About 65% of the radioactivity initially present in the ribosomes was released into the supernatant fraction. An incorporation experiment was carried out in parallel using ribosomes prepared from cells incubated with nonradioactive leucine (Figure 5b). In this experiment 80% of the [^{14}C]leucine incorporated appeared in soluble protein, at 60 minutes. The release of radioactivity was energy dependent (Figure 5a), as would be expected if the release process required the completion of prelabeled polypeptide chains with nonradioactive amino acids.

Two additional experiments of this design were carried out. In one, 35% of the radioactivity in the prelabeled ribosomes was released into the supernatant fraction, in the other 70% was released. (For the incorporation experiments performed in parallel, 45 and 75%, respectively, of the [^{14}C]leucine incorporated appeared in the supernatant fraction.) The failure to obtain a complete release of the radioactivity from prelabeled ribosomes indicates that a fraction of the

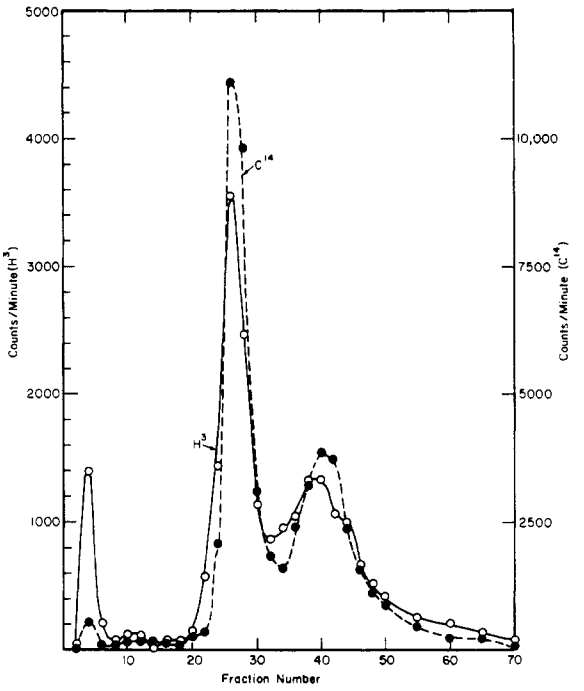

FIGURE 8: Separation of the α- and β-chains of hemoglobin on a carboxymethylcellulose column. Globin was prepared from the 60-minute supernatant fraction of the release experiment with [^{3}H]labeled ribosomes described in the text. The column fractionation was developed using a linear gradient of buffer between 0.2 M formic acid–0.02 M pyridine and 2 M formic acid–0.2 M pyridine. Samples (0.15 ml) from each fraction (1.5 ml) were precipitated with trichloroacetic acid, the precipitates were collected on Millipore filters, and the radioactivity was measured using the scintillation counter.

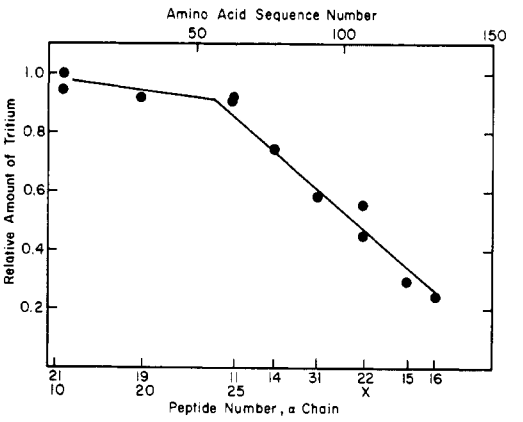

FIGURE 9: Distribution of [^{3}H]leucine among the tryptic peptides of the soluble hemoglobin (α-chain) from the 60-minute cell-free incubation of prelabeled ribosomes. See legend to Figure 1 for details.

ribosomes active in the whole cell do not complete the synthesis of their polypeptide chains in the cell-free system. These "inactive" ribosomes may only be partially impaired, i.e., add a few amino acids to but not complete the total formation of their polypeptide chains. The incorporation experiments showed that some of the radioactivity incorporated was not released from the ribosome fraction.

Reverse-Labeling Patterns. Ribosomes were labeled with [^{3}H]leucine by incubation of the intact cells at 37° for 10 minutes, by which time the amount of radioactivity in the ribosomes had reached the steady-state level. The prelabeled ribosomes were extracted and incubated in the cell-free system (60 minutes at 37°). The amount of ^{3}H in the ribosomal-bound hemoglobin peptides was determined for 0 and 60 minute samples by the method described above. The results are presented in Figures 6 and 7. The data for the two time samples can be plotted on the same graph since the uniformly labeled [^{14}C]leucine hemoglobin was added in proportion to the mass of ribosomes.

In the 0-minute sample, there exists a gradient of radioactivity which suggests the presence in the ribosomes of incomplete polypeptide chains, i.e., hemoglobin chains of differing lengths but each possessing an NH_2 terminus (Dintzis, 1961). It is also evident that the label distribution in the peptide chains remaining in the ribosomes at 60 minutes is similar to that found at 0 minutes, this being true for both the α- and β-chains. This result again suggests a process in which the polypeptide chains that had been released from the ribosomes during incubation were selected at random from the initial population.

The supernatant fraction from a 100,000 × g, 1-hour centrifugation of the 60-minute sample was mixed with hemoglobin uniformly labeled with [^{14}C]leucine. The α- and β-chains were separated by column chromatography (Dintzis, 1961) and the amount of ^{3}H and ^{14}C was compared for each fraction (Figure 8). The ratio of ^{3}H recovered to ^{14}C recovered in the two chains was two-thirds. Thus, about two-thirds of the radioactivity released from the prelabeled ribosomes during cell-free incubation appears in the form of completed hemoglobin chains.

The distribution of the [^{3}H]leucine in the peptides of the α-chain was also determined (Figure 9). It can be seen that there is a gradient of labeling extending through the latter half of the α-chain. It seems that the chains initially in the ribosomes with a length of less than 50 amino acids may not reach the supernatant fraction as complete α-chains. The β-chain sample was lost during electrophoresis of the tryptic digest.

Discussion

The cell-free system employed in this study was first described by Schweet *et al.* (1958). These investigators demonstrated that about 75% of the radioactive protein found in the soluble (100,000 × g, 1-hour supernatant) fraction at the end of incubation was indistinguishable from the main hemoglobin component when frac-

tionated on an ion-exchange column. It can be further concluded from the results presented in our study that the radioactive leucine residues are found in the expected tryptic peptides of the α- and β-chains. This latter result has also been demonstrated using leucyl transfer RNA as the precursor (Bishop *et al.*, 1961).

From the distribution of labeled leucine residues in the completed hemoglobin chains, as well as in the ribosomal-bound chains, it can be concluded that the assembly process occurs by the stepwise addition of amino acids to incomplete polypeptide chains existing in the ribosomes, in the direction of amino to carboxyl end. There was apparently little delay between completion of the chain and its release from the ribosome. This evidence favors the conclusion that the reticulocyte cell-free system synthesizes hemoglobin by the same pathway that is available in the whole cells (Bishop *et al.*, 1960; Dintzis, 1961).

There are important differences, however, between hemoglobin synthesis in this cell-free system and in the whole cell. The initiation of new chains is severely reduced in the former, as was observed also by Bishop *et al.* (1960). Moreover, the initial rate of amino acid incorporation (per unit time) is only about 1% of that found in the reticulocyte. Since the completion of these studies Lamfrom and Knopf (1964) have reported on a cell-free system which synthesizes hemoglobin at 25% the whole cell rate and in which over half of the polypeptide chains made are formed *de novo*. Other investigators have also reported finding a limited amount of cell-free hemoglobin synthesis *de novo* (Hardesty, *et al.*, 1963; Bishop, 1964). Preservation of the polyribosomal structures and alternative methods for fractionation of the components from the intact reticulocyte appear to enhance the level of new chain formation.

In the most active preparations in this study about three leucine residues are incorporated per ribosome and some 75–80% of these are found in completed hemoglobin chains at the end of the incubation. The number of leucine residues per chain of rabbit hemoglobin is not precisely known, but an estimate of one residue in ten would be a good approximation (see Diamond and Braunitzer, 1962, and Naughton and Dintzis, 1962, for the available information). Only half of these leucine residues are labeled, on the average, in the cell-free synthesized product (Figures 1 and 2). Thus it can be calculated that about 1 complete chain is formed for every 3–4 ribosomes present in the cell-free system. In the whole cell, only about one-half of the ribosomes are actively participating in hemoglobin synthesis (Warner *et al.*, 1963) and only one polypeptide chain is produced per active ribosome (Warner and Rich, 1964). It can be concluded, therefore, that about one-half of the ribosomes active in protein synthesis in the whole cell can complete their hemoglobin chains in the cell-free incubation. The presence of inactive ribosomes was indicated above (see Results) from the discrepancy found between the rate of amino acid incorporation per chain and the rate per ribosome and from the failure to obtain complete release of radioactivity from prelabeled ribosomes. Based on the distribution pattern of the labeled leucine residues in the completed hemoglobin chains (Figures 1 and 2) or in the prelabeled ribosomes after cell-free incubation (Figures 6 and 7), it seems that the potentially functional ribosomes from the whole cells are inactivated (partially or totally) in a random manner with regard to the initial length of their incomplete polypeptide chains. The inactivation is probably a result of the procedure employed in the preparation of the ribosomes (Warner *et al.*, 1963).

Acknowledgment

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Isolation of Seryl and Phenylalanyl Ribonucleic Acid Synthetases from Baker's Yeast*

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ABSTRACT: As part of a study of the interaction of proteins and nucleic acid polymers in purified systems a seryl s-RNA synthetase has been isolated from yeast in crystalline form; the enzyme was found to be homogeneous as determined by equilibrium ultracentrifugation. The purification procedure used also yielded a preparation of phenylalanyl s-RNA synthetase of at least 90% homogeneity. The seryl and phenylalanyl s-RNA synthetases have molecular weights (determined by equilibrium ultracentrifugation) of 89,000 and 180,000 respectively; $s_{20,w}$ values (determined by sucrose density gradient centrifugation) of 6.7 and 8.2, respectively; and turnover numbers for catalysis of aminoacyl s-RNA formation of about 50 moles of seryl s-RNA and 100 moles of phenylalanyl s-RNA per minute per mole of enzyme, respectively. The partial purification of the s-RNA synthetases for arginine and leucine from yeast is also described.

The nature and specificity of interaction of proteins and nucleic acid polymers in biological systems are as yet poorly understood. A potentially useful and interesting system for studying this interaction is that involving an amino acid–specific soluble (transfer) RNA (s-RNA) and the corresponding aminoacyl s-RNA synthetase. The synthetases catalyze the following two-step reaction:

$$\text{enzyme} + \text{ATP} + \text{amino acid} \rightleftharpoons \text{enzyme–aminoacyl adenylate} + \text{PP} \quad (1)$$

$$\text{enzyme–aminoacyl adenylate} + \text{s-RNA} \rightleftharpoons \text{aminoacyl s-RNA} + \text{enzyme} + \text{AMP} \quad (2)$$

Purified preparations of certain synthetases (Davie *et al.*, 1956; Van de Ven *et al.*, 1958; Schweet and Allen, 1958; Webster, 1961; Clark and Eyzaguire, 1962; Norris and Berg, 1964) and also of certain amino acid–specific s-RNA species (Holley and Merrill, 1959; Zachau *et al.*, 1961; Tanaka *et al.*, 1962; Ingram and Sjoquist, 1963; Von Ehrenstein and Dais, 1963; Goldstein *et al.*, 1964) have been reported by a number of investigators. Also some data on species specificity and kinetics of aminoacyl s-RNA formation have been obtained with the use of systems containing one of the macromolecular components in purified or partially purified form (Berg *et al.*, 1961; Clark and Eyzaguire, 1962; Bennett *et al.*, 1963; Lagerkvist and Waldenstrom, 1964). However, no study of the interaction of an s-RNA substrate with its specific enzyme has been reported in a system in which both macromolecular components were present in highly purified form.

In recent work in this laboratory, serine-specific s-RNA from yeast has been isolated in essentially pure form (Cantoni *et al.*, 1963). It was decided therefore to attempt to isolate the corresponding aminoacyl s-RNA synthetase in order to study protein–nucleic acid interaction with this system. The present paper describes the isolation of seryl s-RNA synthetase (serine-activating enzyme) from baker's yeast in pure and crystalline form, preparation of a nearly homogeneous phenylalanyl s-RNA synthetase, and also partial purification of the synthetases for arginine and leucine. A later paper will describe studies concerning the interaction of the seryl s-RNA synthetase with its s-RNA substrate.

Experimental

Measurement of Aminoacyl s-RNA Synthetase Activity. For routine assay, reaction mixtures contained

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